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Supplementary Materials for

Overcoming Preexisting Humoral Immunity to AAV Using Capsid Decoys

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The PDF file includes:

Materials and Methods
Fig. S1. AAV empty capsids prevent vector neutralization by anti-AAV NAbs in vitro.
Fig. S2. Large excess of AAV2 empty capsids does not inhibit AAV8-F.IX–mediated live transduction.
Fig. S3. Analysis of hF.IX expression levels and anti-hF.IX IgG levels in nonhuman primates.
Fig. S4. Analysis of capsid-specific humoral and cellular immune responses in nonhuman primates.
Fig. S5. Characterization of the AAV585/8 mutant capsid.
Fig. S6. Vector genome biodistribution in mice receiving an AAV8-F.IX vector formulated in AAV8 or AAV585/8 empty capsids.
Fig. S7. Timing of AAV585/8 empty capsid administration does not have a major influence on hF.IX transgene expression.
Table S1. NAb titers to AAV88 in a cohort of hemophilia B subjects.
Table S2. AAV8-F.IX vector biodistribution in nonhuman primates.
Table S3. Clinical chemistry data over time in nonhuman primates dosed with AAV8-F.IX vectors alone formulated in a 9X excess of AAV8 empty capsids.

SUPPLEMENTARY MATERIALS

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Quantification of circulating antibody:capsid immune complexes

For the quantification of immune complexes in plasma of animals after vector delivery, ELISA plates (Corning Costar) were coated overnight at 4^{o}C with a monoclonal mouse anti-AAV8 antibody developed in our laboratory(*41*) at a concentration of 1μg/ml in carbonate buffer. Plates were then blocked with 0.05% BSA buffer and samples loaded overnight. Detection of the antibody:capsid immune complexes was done with an anti-human IgG Fc horseradish peroxidase conjugated antibody (GE Healthcare). A standard curve was prepared by incubating known amounts of AAV8 capsid with 20μg/ml of IVIg (Grifols Therapeutics) 1 hour at 37^{o}C.

Animal experiments

Animal experiments were approved by the Institutional Animal Care and Use Committees at the Children's Hospital of Philadelphia and Charles River Laboratories Preclinical Services. Male C57BL/6 mice 8 to 10 weeks of age were purchased from Charles Rivers Laboratories. Intravenous immunoglobulin (IVIg, Gamunex) was injected intraperitoneally in a total volume of 200 μl of 1x PBS.

For the nonhuman primate studies, vector was delivered by peripheral vein infusion as previously described(*42*). All experiments in mice were repeated at least twice.

Antibody assays

The in vitro anti-AAV neutralizing antibody assay was based on a previously described protocol(*2*) modified by using an AAV vector expressing the reporter gene luciferase to increase the sensitivity of the assay(*16*). In the assay, the NAb titer corresponds to the reciprocal dilution of the test serum at which 50% inhibition of the luciferase signal is observed compared to the signal of the "virus only" control. The "percent inhibition of undiluted serum" corresponds to the neutralizing activity of undiluted test serum mixed with the reporter gene virus and compared with the signal of the reporter-only control. This number was calculated as follows:

100 - [(undiluted test serum reporter activity – no-reporter background)/(reporter-only control – no-reporter background) X 100].

Factor IX and anti-human F.IX antibody determination

Levels of human F.IX transgene product in mouse plasma were detected with a commercial ELISA kit from Affinity Biologicals. Human F.IX and anti-human F.IX antibodies in rhesus macaque plasma were determined as previously described(*21*).

AAV vector genome copy determination

Real time quantitative PCR (Q-PCR) primers and probes were as follows: for the detection of the AAV-F.IX vector genomes, forward primer 5'-CGAATTCTAGTCGTCGACCACTT-3', reverse primer 5'-CATGTTCACGCGCTGCATA-3', probe 5'-CACAATCTGCTAGCAAAG-3'. The assay was performed as previously described(*21*).

Intracellular imaging and Cytotoxic T lymphocyte (CTL) assay

Image acquisition was performed with an Amnis ImageStreamX instrument (Amnis Corporation). Imaging was performed at a 40x magnification. At least 5000 cells were acquired for each condition. Cells were incubated with untreated or cross-linked AAV2 virus in serum-free medium. After one or four hours, cells were washed twice with 1x PBS and stained. For intracellular staining, $5x10^5$ cells were fixed and permeabilized for 20 min at 4°C then washed with 1X Perm/Wash buffer (BD Bioscience). Cells were then stained with the anti-AAV antibody A20 (Fitzgerald Industries International) together with a rabbit anti-CD71 antibody (Epitomics) or DRAQ5 (Invitrogen). After 30 minutes at room temperature, cells were washed and stained with secondary antibodies.

The CTL assay was previously described(*14, 21*).

Statistical analysis

Statistical analysis was performed with GraphPad Prism 5.0b (Graph Pad Software, Inc). Two-tailed, unpaired t test was used to compare means between treatment groups; p values <0.05 were considered significant.

Fig. S1

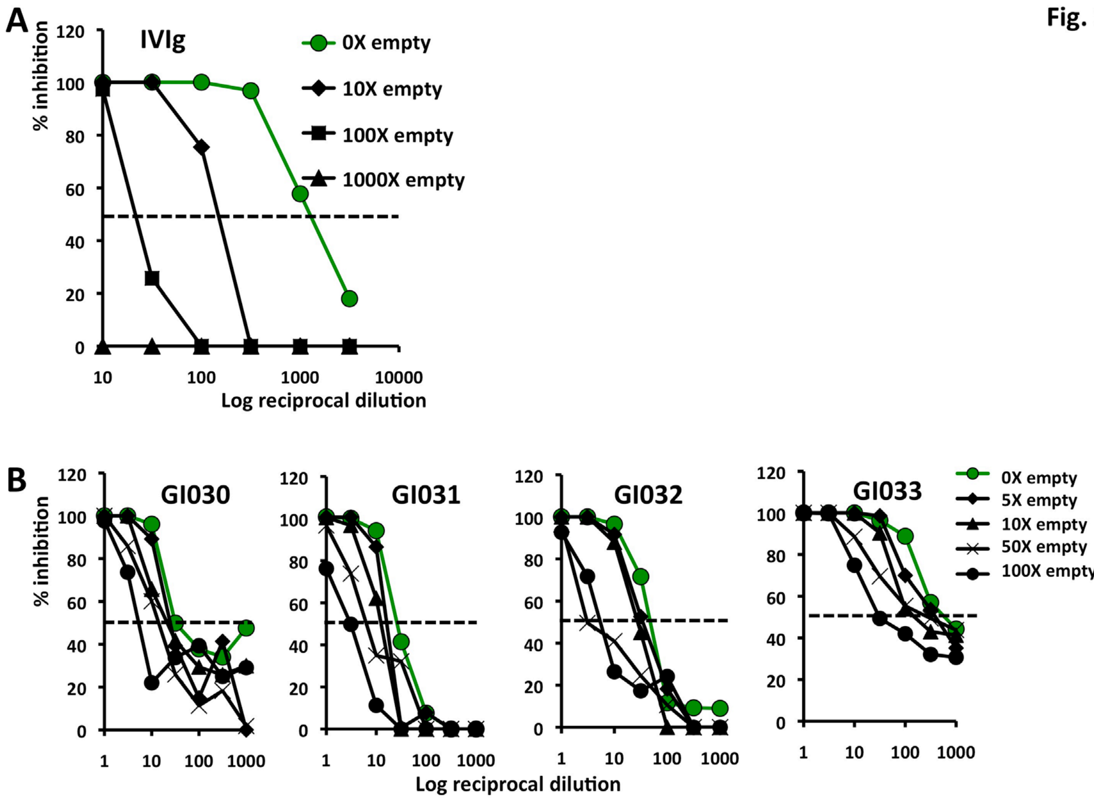

Fig. S1. AAV empty capsids prevent vector neutralization by anti-AAV NAbs in vitro. (**A**) An AAV8 vector expressing luciferase (AAV8-Luciferase, MOI 1x10^3) alone (green line), or in the presence of excess empty AAV8 capsids (10X, 100X, or 1000X the MOI), was incubated with ½ log dilutions of IVIg. % inhibition was determined compared to intensity of the reporter signal for AAV8-Luciferase vector not incubated with IVIg. (**B**) Effect of addition of an excess (5X, 10X, 50X, 100X) of empty AAV8 capsid on the neutralizing activity of serum from human subjects with hemophilia B; the assay was performed as described for IVIg in panel A. Note that the % neutralization for IVIg was tested at dilutions from 1:10 to 1:3000 (panel A, x-axis) while for the hemophilia B samples the % neutralization was tested at dilutions from 1:1 to 1:1000 (panel B, x-axis).

Fig. S2

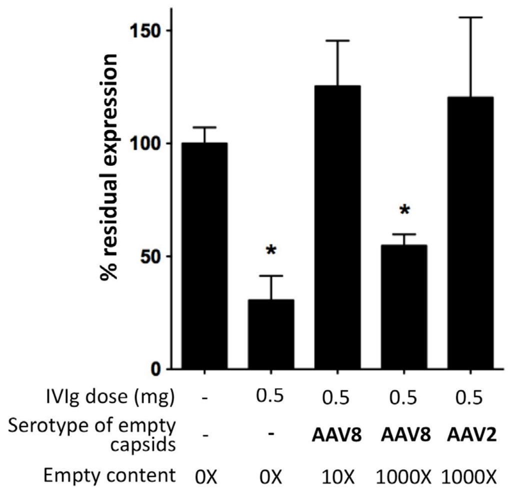

Fig. S2. Large excess of AAV2 empty capsids do not inhibit AAV8-F.IX-mediated live transduction. Male C57BL/6 mice (n=5 per group) were passively immunized with 0.5mg of IVIg or injected with PBS (-) intraperitoneally. 24 hours after, animals received $5x10^9$ vg of an AAV8-F.IX vector alone (-) or formulated with a 10X or 1000X excess of AAV8 empty capsids, or 1000X excess of AAV2 empty capsids. Results are shown as average, error bars represent the standard error of the mean. F.IX levels are expressed as % of F.IX levels in naïve mice receiving the AAV8-F.IX vector only. *, $p<0.05$ vs. vector alone in naïve animals (two-tailed, unpaired t test).

Fig. S3

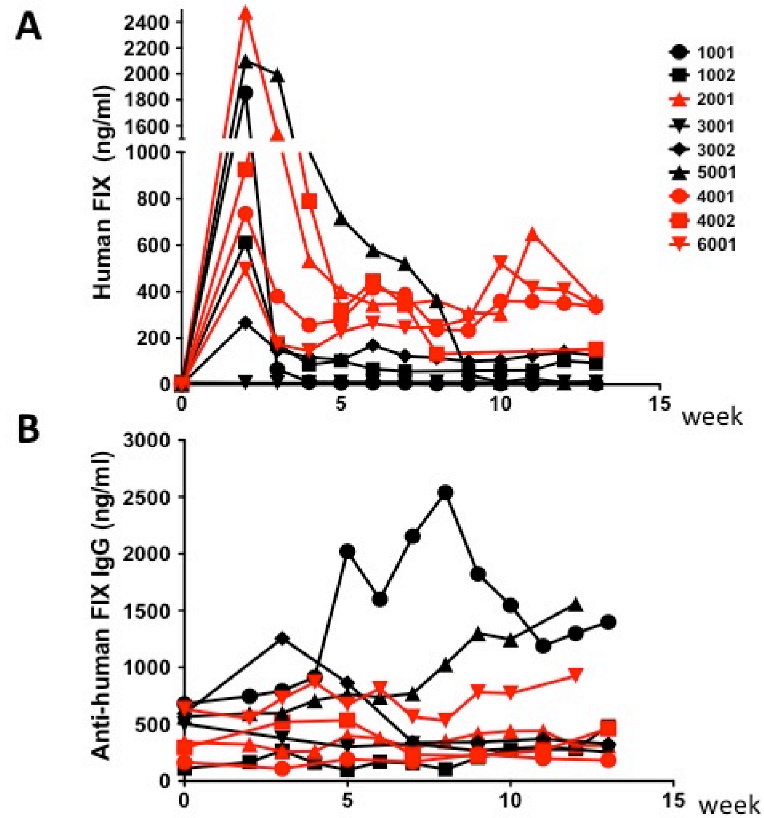

Fig. S3. Time course of human F.IX expression and anti-human F.IX IgG formation in nonhuman primates after AAV8-F.IX vector delivery. (**A**) Human factor IX plasma levels. (**B**) Anti-humans factor IX IgG. Red lines, animals receiving vector formulated in an excess of empty capsids; Black lines, animals receiving vector alone. Animals 1001 and 5001 developed an anti-hF.IX antibody response at week 3 and week 9, respectively. Bethesda assay performed on the samples confirmed that these antibodies were neutralizing.

Fig. S4

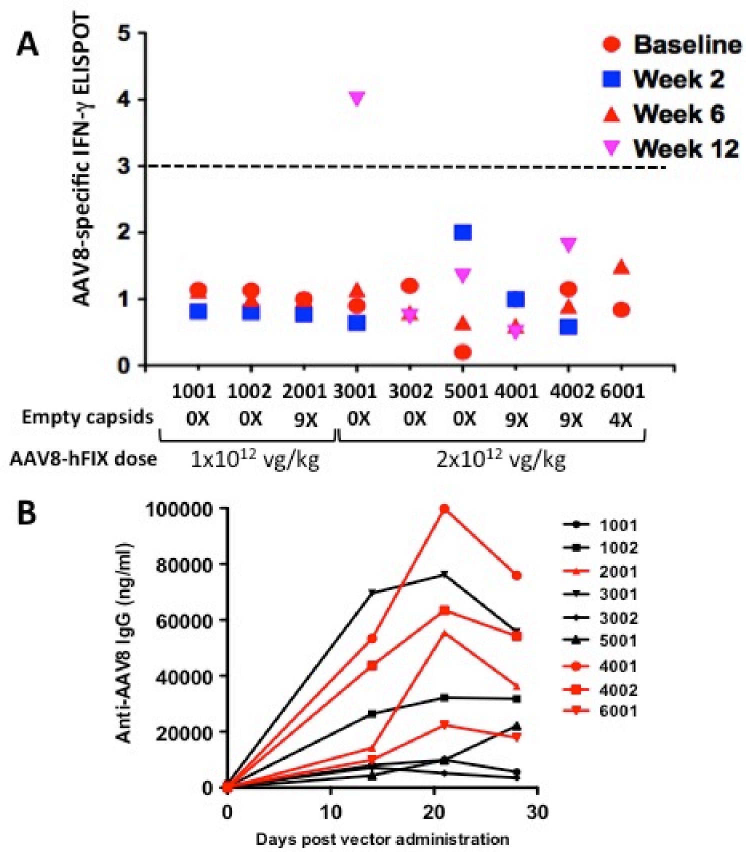

Fig. S4. Analysis of capsid-specific humoral and cellular immune responses in non-human primates. (**A**) AAV8 capsid-specific IFN-γ ELISpot assay on peripheral blood mononuclear cells (PBMC) collected at baseline and at week 2, 6, and 12 post-vector delivery. PMBCs were restimulated overnight with AAV8 peptides on plates coated with an anti-rhesus IFN-γ antibody and reactivity was measured with an anti-rhesus IFN-γ antibody biotin conjugated followed by detection with streptavidin-alkaline phosphatase. Results are shown as fold over medium control. Reactivity above 3 folds the medium control (dashed line) and above 50 spot forming cells per million PBMC was considered positive. One animal, 3001, showed T cell reactivity to the AAV8 capsid at week 12, however the absolute count in this animal was 53 spot forming cells per million PBMC, which is considered low positive. (**B**) Anti AAV8 capsid-specific IgG analysis in serum collected from nonhuman primates at baseline and after vector administration. The average titer of IgG titer at day 28 for animal receiving vector only vs. vector formulated in empty capsids was 23758±21384 vs. 46065±24807 ($p=0.7564$, two-tailed, unpaired t test).

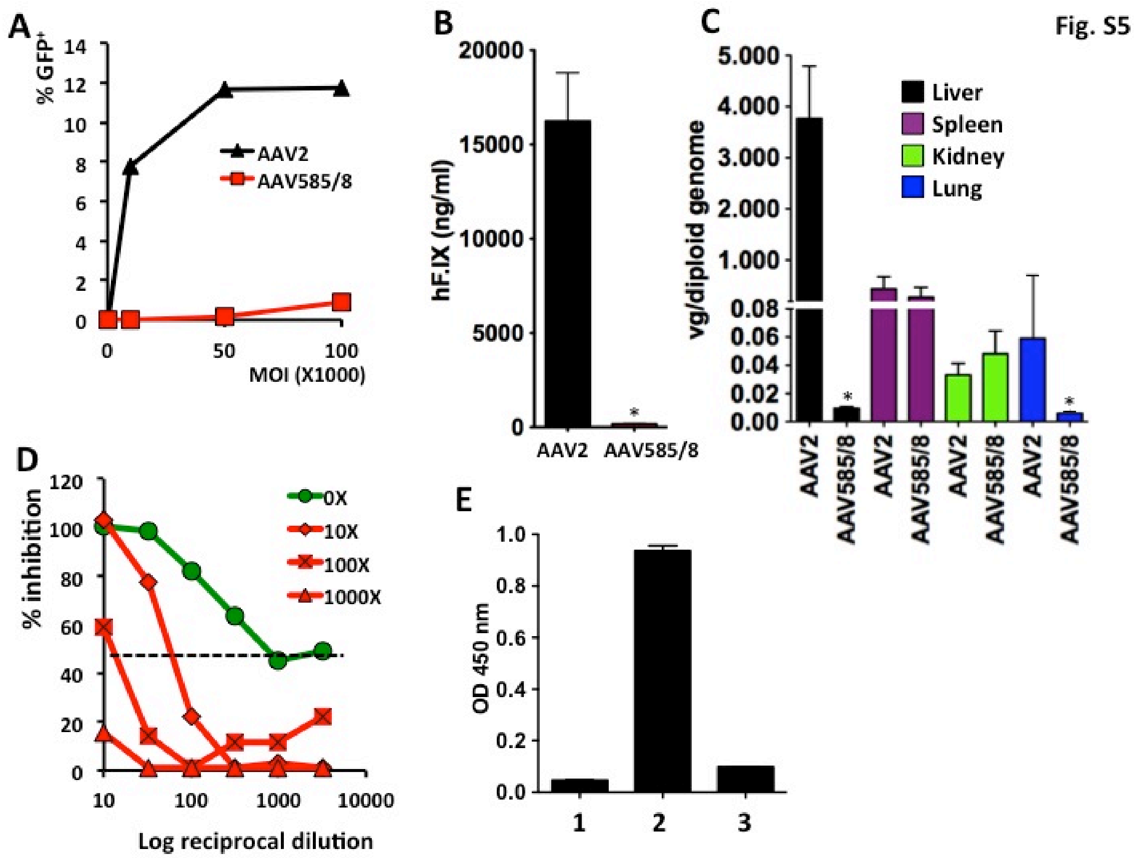

Fig. S5. Characterization of the AAV585/8 mutant capsid. (**A**) Efficiency of transduction in vitro in HEK293 cells. Cells were transduced at increasing multiplicity of infection (MOI) with AAV2 or AAV585/8 vectors expressing GFP. GFP expression was evaluated 24 hours later by flow cytometry. (**B**) Efficiency of transduction in vivo in C57BL/6 mice. Mice (n=4) received $1x10^{11}$ vg of AAV2 or AAV585/8 vectors expressing human F.IX via the tail vein. F.IX transgene product levels in plasma were measured by ELISA 4 weeks post gene transfer. Results are shown as average, error bars represent the standard error of the mean. *, p<0.05 vs. AAV2 (two-tailed, unpaired t test). (**C**) Vector genome biodistribution in mice from (B). Mice were sacrificed at week 8 post gene transfer and DNA from various tissues was collected and analyzed using quantitative real-time PCR (see Methods). Results are shown as average, error bars represent the standard error of the mean. *, $p<0.05$ vs. AAV2 (two-tailed, unpaired t test). (**D**) The AAV585/8 capsid mutant binds anti-AAV antibodies in vitro. An AAV8 vector expressing luciferase (AAV8-Luciferase, MOI $1x10^3$) alone (green line), or in the presence of excess empty AAV585/8 capsids (10X, 100X, or 1000X the MOI, red lines), was incubated with ½ log dilutions of IVIg. % inhibition was determined compared to intensity of the reporter signal for AAV8-Luciferase vector not incubated with IVIg. (**E**) Binding of the anti-AAV2 monoclonal antibody A20 to the AAV585/8 mutant. ELISA plates coated with a 1:500 dilution of A20 antibody were blocked and a $5x10^{10}$ cp/ml of AAV585/8 capsids added to the wells. After an o.n. incubation at 4^{o}C, a 1:500 dilution of IVIg (20μg/ml IgG) was added to the plate and bound antibodies detected with an anti-human IgG antibody conjugated with horseradish peroxidase. 1. AAV585/8 only (no IVIg); 2, AAV585/8 + IVIg; 3, IVIg only.

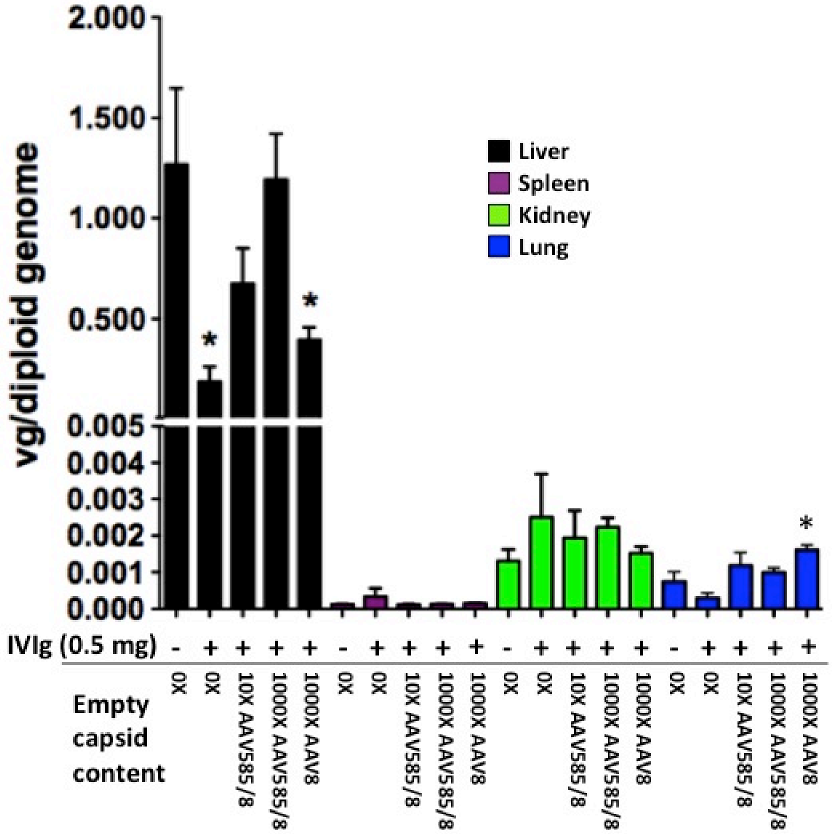

Fig. S6. Vector genome biodistribution in mice receiving an AAV8-F.IX vector formulated in AAV8 or AAV585/8 empty capsids. Male C57BL/6 mice (n=5 per group) were passively immunized with 0.5mg of IVIg (+) or injected with PBS (-) intraperitoneally. 24 hours after, animals received 5x10^9 vg of an AAV8-F.IX vector alone (0X) or formulated with a 10X excess of AAV585/8, 1000X AAV585/8, or 1000X of AAV8 empty capsids. Mice (n=4 or 5) were sacrificed at week 8 post gene transfer and DNA from various tissues was collected and analyzed using quantitative real-time PCR (see Methods). Results are shown as average, error bars represent the standard error of the mean. *, $p<0.05$ vs. naïve mice receiving vector only (two-tailed, unpaired t test).

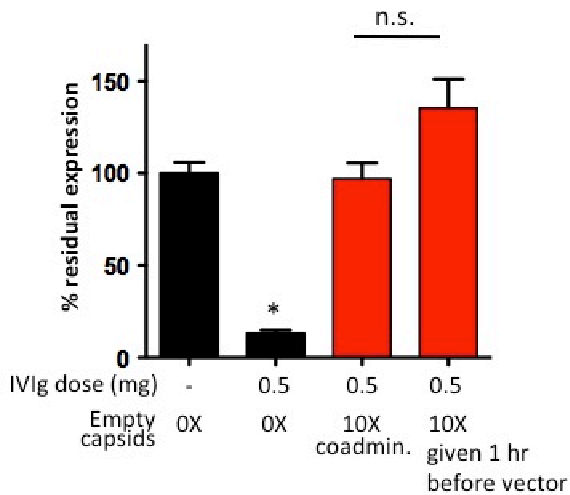

Fig. S7. Timing of AAV585/8 empty capsid administration does not have a major influence on hF.IX transgene expression. Male C57BL/6 mice (n=4 per group) were passively immunized with 0.5mg of IVIg or injected with PBS (-) intraperitoneally. 24 hours after, animals received 5×10^9 vg of an AAV8-F.IX vector alone (0X) or formulated with a 10X excess of AAV585/8 empty capsids (10X coadmin.), or 5×10^{10} cp (10X) of AAV585/8 empty capsids followed by 5×10^9 vg of AAV8-F.IX vector alone one hour later (10X given one hr before vector). Results are shown as average, error bars represent the standard error of the mean. F.IX levels are expressed as % of F.IX levels in naïve mice receiving the AAV8-F.IX vector only. *, $p<0.05$ vs. vector alone in naïve animals (two-tailed, unpaired t test). n.s., not significant, two-tailed, unpaired t test to compare average hF.IX levels of "10X coadmin." vs. "10X given one hr before vector".

Table S1. NAb titers to AAV88 in a cohort of hemophilia B subjects.

Subject ID	Anti-AAV8 NAb
001	1:10
002	1:100
003	1:1
004	1:3
005	1:1
006	1:1000
007	1:300
008	>1:3000
009	1:1
010	1:100
011	1:100
012	1:30
013	1:1
014	1:1
015	1:1
016	1:3
017	1:1
018	1:300
019	1:1
020	1:1
021	1:1
022	1:1
023	1:10
024	1:1
025	1:1
026	1:30
027	1:3
028	1:10
029	1:10
030	1:1

Table S2. AAV8-F.IX vector biodistribution in nonhuman primates.

Each animal received $2x10^{12}$ vg/kg of vector. Animals 4001 and 4002 received a vector formulated in 9X empty capsids. Results are reported as vector genome copy number per diploid genome.
Avg, average of triplicate testing. StDev, standard deviation.

Tissue	Animal ID: 3001		Animal ID: 3002		Animal ID: 4001		Animal ID: 4002	
	Avg	StDev	**Avg**	StDev	**Avg**	StDev	**Avg**	StDev
Liver caudate lobe 1	0.0007	0.0001	0.3014	0.0145	0.0206	0.0173	0.5677	0.0339
Liver caudate lobe 2	0.0065	0.0030	0.1333	0.0199	0.0011	0.0007	0.4514	0.0312
Liver left lateral lobe 1	0.0112	0.0057	0.1990	0.0338	0.0713	0.0286	0.0163	0.0079
Liver left lateral lobe 2	0.0056	0.0042	0.1086	0.0112	0.0454	0.0121	0.6604	0.0196
Liver right lateral lobe 1	0.0379	0.0054	0.1934	0.0065	0.0506	0.0105	0.5458	0.0160
Liver right lateral lobe 2	0.0047	0.0031	0.0087	0.0028	0.0599	0.0292	0.3821	0.0378
Liver left medial lobe 1	0.0049	0.0025	0.0128	0.0051	0.2285	0.0198	0.5091	0.0436
Liver left medial lobe 2	0.0057	0.0040	0.1437	0.0128	0.1451	0.0186	0.0109	0.0035
Liver right medial lobe1	0.0018	0.0004	0.0341	0.0128	0.1202	0.0129	0.6715	0.0373
Liver right medial lobe 2	0.0077	0.0056	0.0056	0.0022	0.0128	0.0012	0.6134	0.0210
Liver quadrate lobe 1	0.0051	0.0018	0.0160	0.0047	0.1629	0.0215	0.7107	0.0377
Liver quadrate lobe 2	0.0030	0.0013	0.0294	0.0086	0.0170	0.0069	0.0130	0.0054
Average Liver	**0.0079**	0.0098	**0.0988**	0.0969	**0.0780**	0.0710	**0.4294**	0.2673
Brain	**0.0093**	0.0048	**0.0254**	0.0233	**0.0173**	0.0124	**0.0377**	0.0063
Kidney	**0.0265**	0.0175	**0.0959**	0.0624	**0.0115**	0.0038	**0.0091**	0.0016
Spleen	**0.0322**	0.0203	**0.0273**	0.0092	**0.0099**	0.0060	**0.0310**	0.0196
Thymus	**0.0136**	0.0056	**0.0000**	0.0000	**0.0000**	0.0000	**0.0333**	0.0076
Testes	**0.0155**	0.0086	**0.1030**	0.1204	**0.0259**	0.0073	**0.0079**	0.0015
Lung	**0.0388**	0.0175	**0.0013**	0.0011	**0.0790**	0.0580	**0.1120**	0.0380
Diaphragm	**0.1995**	0.1130	**0.1194**	0.0771	**0.1267**	0.0354	**0.1458**	0.0816
Psoas	**0.0919**	0.0239	**0.2118**	0.0549	**0.0812**	0.0143	**0.1199**	0.0405
Heart	**0.0083**	0.0058	**0.0740**	0.0478	**0.0150**	0.0164	**0.0347**	0.0177

Table S3. Clinical chemistry data over time in nonhuman primates dosed with AAV8-F.IX vectors alone formulated in a 9X excess of AAV8 empty capsids. Animals 1001 and 1002 received 1x10^{12} vg/kg of AAV8-F.IX vector; animal 2001 received 1x10^{12} vg/kg of AAV8-F.IX vector formulated in 9X empty capsids; animals 3001 and 3002 received 2x10^{12} vg/kg of AAV8-F.IX vector; animals 4001 and 4002 received 2x10^{12} vg/kg of AAV8-F.IX vector formulated in 9X empty capsids.

Animal Number	Day Number	ALT U/L	AST U/L	ALP U/L	GGT U/L	LD U/L	TBili mg/dL	BUN mg/dL	Crea mg/dL	Ca mg/dL	Phos mg/dL	TP g/dL
1001	-5	53	76	514	79	391	0.2	21	0.5	9.3	5.3	6.9
	2	51	64	591	74	552	0.3	23	0.6	9.4	5.0	7.3
	10	25	36	485	72	220	0.2	19	0.5	9.4	5.5	7.3
	17	32	47	495	76	508	0.2	24	0.7	9.7	5.7	7.4
	24	28	29	495	69	268	0.2	29	0.6	9.4	5.6	7.7
	31	26	25	466	69	199	0.1	36	0.6	9.3	5.1	7.5
	38	34	33	491	78	422	0.2	44	0.7	9.5	6.2	7.8
	45	32	27	526	78	265	0.1	30	0.7	8.9	5.9	7.6
	52	26	25	557	82	248	0.1	26	0.7	9.5	6.0	7.8
	59	30	59	538	82	871	0.2	31	0.8	9.4	6.0	7.6
	66	29	29	507	79	384	0.1	45	0.7	9.4	5.2	7.5
	73	28	24	533	79	225	0.2	25	0.7	8.8	4.9	7.4
	80	27	25	488	74	281	0.1	44	0.8	9.5	5.9	7.5
	87	27	22	518	71	198	0.1	23	0.8	9.0	5.7	7.4
1002	-5	27	26	515	68	227	0.2	22	0.5	10.0	5.2	6.9
	2	47	49	787	100	516	0.3	20	0.4	9.8	6.4	7.4
	10	40	34	430	58	277	0.2	25	0.6	10.1	7.2	7.3
	17	38	33	445	58	306	0.3	27	0.5	9.8	5.9	6.8
	24	36	32	409	56	324	0.3	28	0.6	9.8	6.8	7.2
	31	35	36	389	56	353	0.2	29	0.6	10.3	6.2	7.7
	38	35	42	353	56	417	0.3	34	0.6	9.6	6.8	7.4
	45	38	31	342	54	306	0.2	28	0.6	9.7	5.9	7.4
	52	44	38	388	57	283	0.2	30	0.6	9.7	6.3	7.3
	59	31	30	375	62	233	0.3	29	0.7	10.1	6.2	7.6
	66	34	33	394	63	261	0.2	33	0.7	9.8	6.1	7.2
	73	33	32	444	68	309	0.2	30	0.8	9.7	5.9	7.6
	80	31	28	392	64	255	0.2	26	0.7	9.7	6.4	7.4
	87	34	26	394	60	236	0.2	24	0.8	10.4	6.3	7.5
2001	-5	80	48	675	108	421	0.2	24	0.4	9.8	5.5	6.8
	2	83	50	503	62	399	0.4	26	0.5	9.4	6.6	7.2
	10	28	25	633	99	184	0.2	18	0.4	9.8	6.1	7.3
	17	32	25	631	97	177	0.2	19	0.4	10.2	6.4	7.3
	24	32	26	578	92	195	0.2	22	0.4	10.0	6.0	7.3

	31	28	25	587	97	190	0.1	20	0.4	10.0	6.2	7.5
	38	30	24	557	103	225	0.1	20	0.5	10.0	6.2	7.7
	45	30	24	488	91	229	0.2	22	0.4	9.3	5.8	7.2
	52	30	23	489	89	173	0.1	23	0.5	9.7	6.1	7.5
	59	34	26	487	90	177	0.2	22	0.6	9.8	5.3	7.6
	66	25	20	460	85	166	0.1	22	0.5	9.7	5.3	7.2
	73	25	21	512	89	180	0.2	23	0.5	9.2	5.0	7.4
	80	32	23	452	89	204	0.1	21	0.6	9.8	5.2	7.6
	87	27	21	503	86	172	0.1	19	0.5	9.7	6.5	7.4
3001	-2	50	27	568	79	349	0.2	17	0.6	9.4	7.7	7.8
	2	95	134	475	68	1140	0.2	12	0.5	9.4	4.4	7.2
	9	56	25	507	75	466	0.2	15	0.6	9.9	8.2	7.7
	17	36	32	490	86	456	0.2	19	0.6	10.1	8.4	8.0
	24	35	26	520	81	441	0.2	17	0.6	10.1	7.5	7.6
	31	42	23	578	89	293	0.2	13	0.7	10.1	7.8	8.2
	36	43	20	578	88	318	0.2	17	0.8	10.0	6.5	8.1
	45	41	47	535	83	735	0.2	18	0.7	10.6	7.1	7.6
	52	39	21	468	82	240	0.2	18	0.7	10.1	6.9	7.5
	59	37	22	486	84	261	0.1	15	0.8	9.8	7.1	8.1
	65	36	35	447	82	533	0.1	16	0.7	9.8	6.6	7.9
	73	31	26	480	88	400	0.1	16	0.7	10.3	8.1	8.0
	80	36	23	539	90	306	0.1	14	0.7	9.9	7.1	8.0
	87	30	24	492	82	333	0.1	15	0.8	9.2	7.5	7.4
3002	-2	279	596	499	50	2308	0.3	17	0.7	10.1	7.0	7.5
	2	218	112	429	45	459	0.2	15	0.6	10.0	3.9	7.5
	9	70	46	452	49	396	0.2	20	0.7	10.6	6.6	8.0
	17	43	32	478	53	312	0.2	20	0.7	10.3	6.7	7.8
	24	38	36	526	56	291	0.2	23	0.7	10.9	7.0	7.9
	31	42	39	512	57	220	0.2	24	0.7	10.1	6.2	7.6
	36	43	31	570	64	240	0.3	21	0.7	10.3	6.4	8.2
	45	39	31	527	60	348	0.2	23	0.7	10.8	5.0	7.4
	52	39	32	518	64	227	0.3	22	0.7	10.3	6.0	7.8
	59	37	34	543	66	213	0.3	24	0.8	10.4	7.0	8.3
	65	43	73	523	63	625	0.4	21	0.8	10.1	7.1	7.6
	73	40	43	538	64	353	0.2	24	0.7	10.5	6.5	7.8
	80	45	41	581	66	347	0.2	20	0.8	10.1	8.8	7.9
	87	54	43	518	59	428	0.3	22	0.7	9.7	6.7	7.7
4001	-2	54	35	713	55	293	0.2	21	0.7	9.5	5.4	7.9
	2	192	253	592	46	2301	0.3	13	0.6	9.8	3.5	7.2
	9	69	40	687	49	465	0.2	16	0.7	10.3	7.3	7.8
	17	43	45	578	50	459	0.2	25	0.7	10.1	5.5	7.6
	24	50	59	634	50	607	0.2	23	0.7	10.2	5.3	7.4
	31	45	39	660	52	329	0.2	22	0.7	9.8	5.0	7.5
	36	47	37	712	54	333	0.2	20	0.7	10.0	5.0	7.3

	45	57	39	736	57	337	0.2	15	0.7	10.4	5.7	7.1
	52	51	34	630	59	281	0.3	21	0.7	10.1	5.5	7.3
	59	57	38	569	56	278	0.2	20	0.6	9.8	4.7	7.3
	65	49	35	583	55	264	0.2	18	0.6	9.5	5.5	7.2
	73	63	44	667	61	327	0.2	18	0.6	9.8	5.6	7.2
	80	56	50	766	69	410	0.2	17	0.7	9.7	5.2	7.3
	87	62	44	685	64	429	0.2	23	0.7	9.8	5.6	7.2
4002	-2	32	24	746	76	247	0.2	16	0.8	9.6	5.9	7.3
	2	47	91	689	63	769	0.4	17	0.8	9.4	5.2	6.8
	9	27	31	712	67	341	0.2	19	0.8	9.6	6.1	7.4
	17	29	33	638	71	310	0.2	21	0.8	10.0	6.7	7.3
	24	24	30	697	73	236	0.2	20	0.8	10.3	5.8	7.3
	31	25	27	732	75	199	0.2	17	0.8	9.7	5.7	7.5
	36	29	33	729	78	299	0.2	17	0.9	10.2	6.6	7.7
	45	74	39	728	82	222	0.2	20	0.9	10.2	6.0	7.3
	52	52	41	665	78	301	0.2	19	0.9	9.9	6.3	7.2
	59	36	31	717	78	178	0.2	16	0.8	9.7	5.6	7.4
	65	33	52	705	76	591	0.2	20	0.9	9.8	5.2	7.4
	73	27	30	685	76	227	0.2	18	0.7	9.6	5.6	7.2
	80	30	50	711	79	426	0.3	21	0.9	9.6	6.8	6.9
	87	35	29	684	72	224	0.2	14	0.7	9.3	5.5	6.8

Animal Number	**Day Number**	**Alb g/dL**	**Glob g/dL**	**A:G ratio**	**Glucose mg/dL**	**Chol mg/dL**	**Trig mg/dL**	**Na mEq/L**	**K mEq/L**	**Cl mEq/L**	**CO2 mEq/L**
1001	-5	3.6	3.3	1.1	108	114	66	142	4.3	108	18
	2	3.8	3.5	1.1	68	107	68	142	4.3	108	19
	10	3.7	3.6	1.0	70	118	60	141	3.8	109	16
	17	3.9	3.5	1.1	84	126	77	145	3.9	108	17
	24	3.7	4.0	0.9	92	125	70	145	4.0	105	18
	31	3.4	4.1	0.8	88	107	104	143	4.0	108	14
	38	3.6	4.2	0.9	112	124	95	144	4.6	105	15
	45	3.4	4.2	0.8	126	118	82	142	4.8	105	17
	52	3.6	4.2	0.9	78	130	80	147	4.5	108	18
	59	3.5	4.1	0.9	77	122	257	144	4.8	109	10
	66	3.5	4.0	0.9	97	117	100	140	4.2	108	14
	73	3.4	4.0	0.9	83	114	98	143	3.7	109	13
	80	3.2	4.3	0.7	119	109	88	139	4.8	107	12
	87	3.3	4.1	0.8	85	116	54	143	3.9	109	17
1002	-5	4.4	2.5	1.8	94	159	84	143	4.2	108	22
	2	4.6	2.8	1.6	56	165	63	144	3.8	108	22
	10	4.8	2.5	1.9	81	154	78	147	4.0	107	14
	17	4.6	2.2	2.1	49	138	106	146	4.0	109	21
	24	4.5	2.7	1.7	93	168	65	145	4.6	110	17

	31	4.4	3.3	1.3	62	142	64	148	4.3	108	19
	38	4.5	2.9	1.6	47	152	114	148	3.6	108	17
	45	4.4	3.0	1.5	94	137	66	144	4.5	108	18
	52	4.6	2.7	1.7	58	149	76	146	4.3	112	17
	59	4.6	3.0	1.5	68	159	56	146	5.0	111	16
	66	4.5	2.7	1.7	89	152	59	146	4.4	111	17
	73	4.7	2.9	1.6	42	144	82	146	4.0	109	21
	80	4.5	2.9	1.6	66	153	60	146	4.1	107	21
	87	4.6	2.9	1.6	72	161	66	149	4.9	110	24
2001	-5	4.3	2.5	1.7	107	162	56	142	4.0	106	23
	2	4.6	2.6	1.8	42	145	53	143	3.9	109	17
	10	4.5	2.8	1.6	74	181	56	144	3.7	109	19
	17	4.8	2.5	1.9	73	176	62	145	4.1	108	22
	24	4.6	2.7	1.7	92	176	63	145	3.6	108	22
	31	4.4	3.1	1.4	81	163	79	145	3.7	107	21
	38	4.6	3.1	1.5	79	179	74	146	3.6	107	22
	45	4.4	2.8	1.6	83	158	83	144	3.3	107	25
	52	4.8	2.7	1.8	68	189	86	146	3.6	108	24
	59	4.8	2.8	1.7	87	197	87	148	3.2	108	19
	66	4.8	2.4	2.0	91	185	68	146	3.5	111	22
	73	4.5	2.9	1.6	59	173	93	142	3.3	112	21
	80	4.6	3.0	1.5	93	177	79	145	3.9	107	22
	87	4.5	2.9	1.6	64	200	62	146	3.6	108	25
3001	-2	4.4	3.4	1.3	56	143	99	146	4.2	105	19
	2	3.9	3.3	1.2	97	124	55	145	4.2	110	23
	9	4.4	3.3	1.3	98	159	66	149	4.9	106	21
	17	4.6	3.4	1.4	111	160	95	150	4.8	108	14
	24	4.2	3.4	1.2	83	145	70	150	5.3	107	21
	31	4.5	3.7	1.2	97	167	61	152	5.0	112	12
	36	4.9	3.2	1.5	99	173	86	149	4.8	107	19
	45	4.2	3.4	1.2	84	146	90	151	6.0	108	19
	52	4.5	3.0	1.5	112	146	62	147	4.4	106	15
	59	4.6	3.5	1.3	130	153	63	150	4.4	107	16
	65	4.5	3.4	1.3	94	159	71	148	4.3	105	21
	73	4.7	3.3	1.4	136	164	58	150	5.2	111	5
	80	4.5	3.5	1.3	86	153	57	151	5.1	109	18
	87	4.5	2.9	1.6	133	144	52	145	3.9	107	11
3002	-2	4.6	2.9	1.6	77	122	122	147	4.2	109	20
	2	4.6	2.9	1.6	69	131	100	147	3.7	105	29
	9	4.9	3.1	1.6	84	160	111	151	4.5	105	21
	17	5.0	2.8	1.8	106	148	84	147	4.1	108	13
	24	4.9	3.0	1.6	81	150	117	152	4.3	104	18
	31	4.6	3.0	1.5	93	135	82	149	3.8	105	21
	36	5.3	2.9	1.8	76	155	82	149	3.5	105	20

	45	4.6	2.8	1.6	76	130	270	149	4.1	105	24
	52	5.1	2.7	1.9	78	145	94	149	3.9	105	21
	59	5.1	3.2	1.6	89	141	142	151	3.5	104	19
	65	4.8	2.8	1.7	58	132	126	147	4.4	106	16
	73	4.9	2.9	1.7	84	138	97	151	4.6	106	20
	80	4.8	3.1	1.5	80	135	100	153	5.4	109	17
	87	4.9	2.8	1.8	77	135	105	148	3.7	106	23
4001	-2	4.6	3.3	1.4	90	157	98	146	3.9	108	18
	2	4.3	2.9	1.5	88	145	57	147	4.1	109	30
	9	4.8	3.0	1.6	84	181	81	152	5.1	108	22
	17	4.8	2.8	1.7	108	161	89	146	3.9	106	22
	24	4.6	2.8	1.6	118	157	110	145	4.5	107	17
	31	4.8	2.7	1.8	103	161	94	147	4.3	108	24
	36	5.0	2.3	2.2	100	170	80	145	4.5	109	19
	45	4.6	2.5	1.8	123	139	136	148	3.9	108	22
	52	4.9	2.4	2.0	99	155	92	148	4.4	109	20
	59	4.6	2.7	1.7	101	158	102	147	4.5	111	19
	65	4.6	2.6	1.8	92	163	88	147	3.6	108	20
	73	4.6	2.6	1.8	84	154	85	149	4.3	109	22
	80	4.7	2.6	1.8	89	142	81	149	4.0	109	19
	87	4.7	2.5	1.9	87	147	86	152	4.4	110	22
4002	-2	4.3	3.0	1.4	83	131	62	147	3.7	110	19
	2	4.2	2.6	1.6	105	109	44	147	4.3	110	22
	9	4.4	3.0	1.5	94	140	93	146	4.0	104	22
	17	4.4	2.9	1.5	93	147	102	147	3.9	105	24
	24	4.4	2.9	1.5	100	141	148	153	4.5	107	21
	31	4.5	3.0	1.5	84	149	96	148	3.6	106	24
	36	4.8	2.9	1.7	116	163	94	153	4.1	107	20
	45	4.3	3.0	1.4	111	139	70	152	4.3	108	18
	52	4.7	2.5	1.9	97	136	88	149	4.6	112	13
	59	4.4	3.0	1.5	100	141	73	146	3.8	110	18
	65	4.6	2.8	1.6	75	133	84	151	3.9	108	21
	73	4.4	2.8	1.6	91	137	73	145	3.8	109	21
	80	4.3	2.6	1.7	70	133	73	150	4.1	108	21
	87	4.4	2.4	1.8	88	130	53	146	4.1	111	21

ALT: alanine aminotransferase
AST: aspartate aminotransferase
ALP: alkaline phosphatase
GGT: gamma-glutamyl transferase
LD: lactate dehydrogenase
TBili: total bilirubin

BUN: blood urea nitrogen
Crea: creatinine
Ca: calcium
Phos: phosphorous
TP: total protein
Alb: albumin
Glob: globulin
A:G: albumin to globulin ratio
Chol: cholesterol
Trig: triglycerides